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DECLARATION OF JAMES M. ZAVISLAN
SUBMITTED UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, James M. Zavislan, currently Associate Professor at the University of Rochester and formerly Vice President for Technology and Chief Technology Officer of Lucid, Inc., assignee of the above-identified application, and one of the inventors of the above referenced application, hereby declare that:

I visited Dr. Koester on October 16, 2000 at the Columbia Presbyterian Medical Center. While visiting Dr. Koester, I saw in the Ophthalmology clinic a specular microscope that Dr. Koester said was based on the scanning mirror microscope instrument described in his 1980 Applied Optics paper entitled "Scanning mirror microscope with optical sectioning characteristics: applications in ophthalmology" (Appl. Opt. Vol. 19, No. 11, 1980). Attached is a picture of the same instrument design that I saw on my October 16, 2000 visit. I have annotated this picture with letters showing the components of the instrument. The instrument was integrated in a fixed platform where the patient placed their head in a chin rest and forehead band, which is identical to that used in slit lamp examinations. The patient was seated on one side of the instrument and the clinician conducting the examination sat on the opposite side. The instrument had a fixed platform (C) mounted to a table (D) that supported the patient interface (A) and a nominally rectangular box (B) that presumably contained the specular microscope. This

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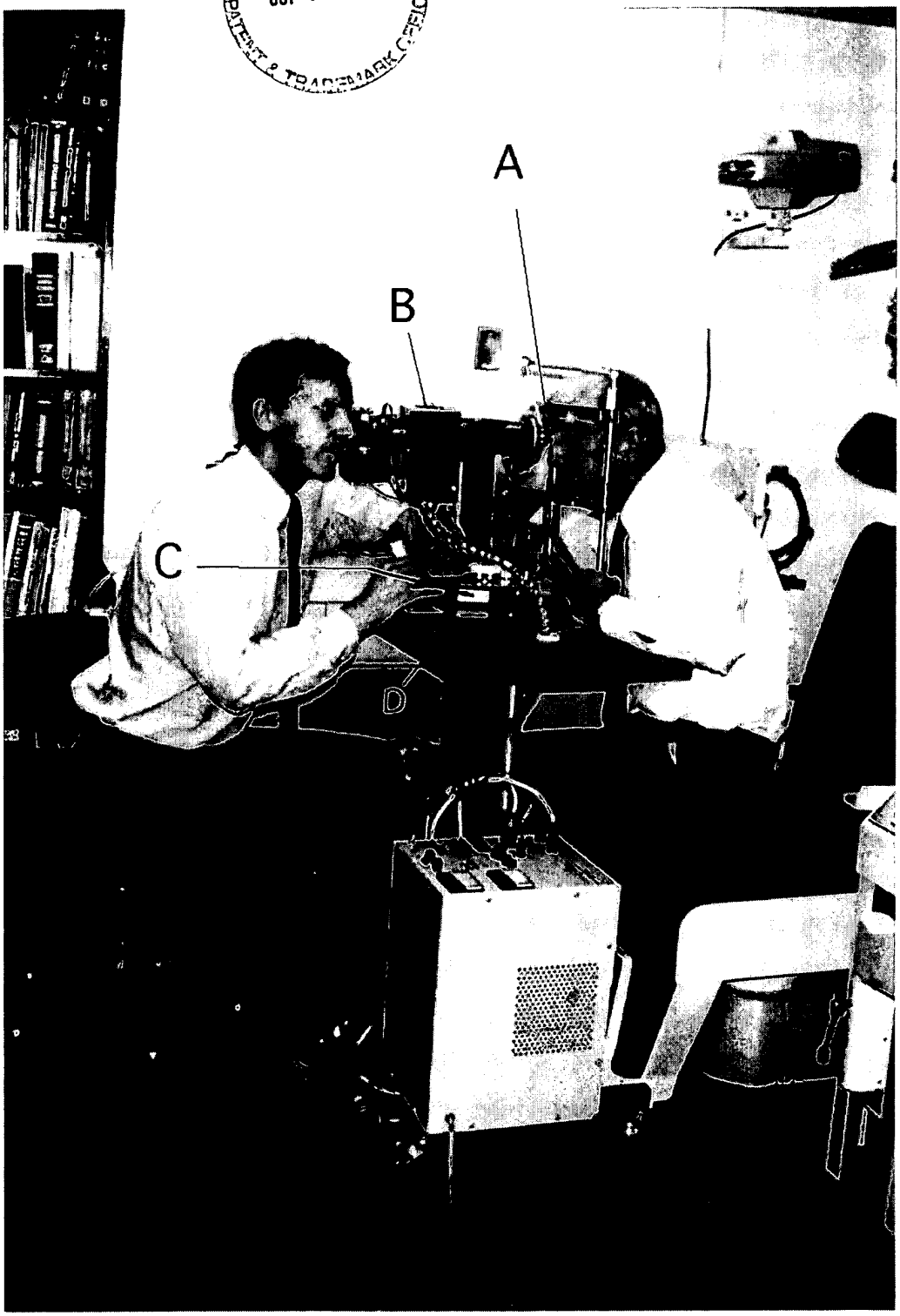
box (B) could not be separated from the supporting platform (C). This box (B) was too large to be hand-held. Thus, the scanning mirror microscope instrument described in Dr. Koester's 1980 Applied Optics paper was too large and bulky to be handheld by a clinician conducting an examination with the instrument.

I further declare that all statements made here in of my own knowledge are true and that all statements made on information and belief are believed to be true; and, further, that the statement were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 27 Sept. 2004

James M. Zavislan

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Wide Field Specular Microscopy

Clinical and Research Applications

CHARLES J. KOESTER, PhD, CALVIN W. ROBERTS, MD, ANTHONY DONN,MD, FRANK B. HOEFLE, MD

Abstract: Through the use of a scanning mirror system, the field of view has been expanded to as much as 0.9 mm in diameter. Both contact and non-contact specular microscopy are possible, each having advantages. In a research application, wound healing in rabbit endothelium is documented by time lapse photography. The study demonstrates the capability to return to an area of interest provided there is a landmark. Clinical applications and advantages over conventional specular microscopy are discussed. [Key words: corneal endothelium, endothelium, non-contact specular microscopy, specular microscopy, wound healing.] Ophthalmology 87:849–860, 1980

Specular microscopy, introduced by Maurice[1] in 1968, has been increasingly used in connection with corneal disease, intraocular surgery, and the study of endothelial cell function. Clinical adaptations of the microscope have been developed by Laing et al[2] and Bourne and Kaufman.[3]

From Columbia University, College of Physicians and Surgeons, New York.

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Reprint request to Charles J. Koester, Ph D, Department of Ophthalmology, Columbia University, College of Physicians and Surgeons, 635 West 165th Street, New York, NY 10032.

Dr. Koester has a proprietary interest in the wide field specular microscope (US Patent 4,170,398 and others pending). The other authors have no proprietary interest in the microscope. The instrument is not yet commercially available.

The problems discussed in this paper are the relatively narrow field of view obtained with the conventional specular microscope, and the non-uniformity of image contrast. The narrow field not only severely limits the number of contiguous cells which can be examined and counted, it also makes relocation of an area of interest on the endothelium very difficult.

An additional problem has been the necessity for contact to the cornea in order to obtain high magnification images. Non-contact instruments have been reported by Holm,[4] and by McCarey,[5] using somewhat lower magnification. For clinical applications, there are obvious advantages to non-contact operation, particularly if the field size is comparable to that of the contact type.

The optical challenge associated with viewing and photographing the endothelial cell layer can be summarized as follows. While the cells are readily seen by specular reflection, the reflection coefficient at the interface between the cells and the aqueous is estimated to be only about 0.02%, a factor of at least 100 less than

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that at the interface between the tear layer and air. Even if contact with the cornea is made, for example, by the glass surface of a dipping cone objective, the reflection coefficient at this interface is about 0.24%, 12 times that of the endothelial surface. The problem, therefore, is to form an image of the endothelial cell layer without any superimposed stray light arising from the anterior surface reflection. It is also desirable to minimize the effect of any scattering that occurs in the stroma, particularly in the case of edematous corneas.

The conventional specular microscope solves these problems by imaging a narrow illuminated slit on the endothelium, which is in turn imaged on the film or eyepiece focal plane. The goals of the present work were to retain the advantages achieved by the Maurice design while increasing the field of view and achieving more uniform contrast across the field, and to realize wide field specular microscopy in a non-contact mode.

METHODS

The principle of the scanning mirror microscope and the performance of a research model have been described previously.[6] Fig 1 is the optical layout of the clinical model. As in the conventional design, an illuminated slit S_1 is imaged on the endothelial cell layer by half of an objective L_3. The specularly reflected light is accepted by the other half of objective L_3, is reflected by a second facet of mirror M_1, and is brought to a focus at a second slit S_2. Slit S_2 is adjusted to be conjugate to the illuminated strip on the endothelium. The wide field is obtained by rotating mirror M_1 about an axis perpendicular to the diagram in an oscillatory manner with an excursion of a few degrees. This causes the image of slit S_1 to scan back and forth across the endothelial surface. Because of the second reflection from mirror M_1, the light returning from the cornea forms a stationary image of illuminated slit S_1 at slit S_2. The function of slit S_2 is to eliminate from the imaging bundle all light reflected from the anterior corneal surface as well as scattered light from regions of the cornea anterior to the endothelium. With certain exceptions noted below, only the light coming from the illuminated strip of the endothelium passes through slit S_2. This light is then reflected by mirrors $M_{3,4}$ to the third facet of mirror M_1. The oscillatory rotation of mirror M_1 causes the image of slit S_2 to be scanned across the final image plane F, thus laying down the final image strip by strip. Because mirror M_1 oscillates at about 1000 Hz, the complete image is reconstructed 2000 times per second, and therefore appears continuous to the observer.

Two light sources are provided, the 75W Xe arc (A) for visual use of the instrument, the flash lamp (FL) for photography. Light from the Xe arc is imaged onto and transmitted through the flash lamp, so that no change in the optical system is needed when switching from observation to photography.

Fig 2 illustrates in detail the relationship between illumination rays and imaging rays in the object space of the scanning mirror microscope. The illumination rays occupy the space between the rays with the downward pointing arrows, and the image of slit S_1 is at S. The imaging rays, those that will be able to pass through slit S_2, are contained between the rays with the upward pointing arrows. Only in the diamond-shaped cross-hatched area can any light be scattered or reflected from the illumination bundle into the imaging bundle. As the image S is scanned back and forth, an optical section will be created in the specimen, lying between planes B and B'. Any reflecting or scattering material outside this optical section, such as in plane A, will not contribute light to the image.

The thickness of the optical section is seen from Fig 2 to be 2h. By the geometry of the diamond-shaped area, 2h is determined by the width, w, of the slit image and by the angle, θ_n, of the ray with the minimum inclination. The relation is

$$\frac{w}{2h} = \tan\theta_n \qquad (1)$$

It can be seen from equation 1 and from the geometry of Fig 2 that if the optical section thickness, 2h, is to be small, the slit width, w, must be small. Also, the minimum angle θ_n of illumination and imaging rays must not be zero, and should be as large as is feasible.

The method for controlling the angle θ_n is shown in Fig 3. An opaque strip is placed at the second focal plane of objective L_3. The width of the strip determines the angle θ_n. The selection of the optimum values for the angle θ_n and the slit image width w are discussed by Koester.[6]

The optical sectioning principle can be utilized to achieve non-contact specular microscopy. The geometry is indicated in Fig 4. If the height, h, of the overlap region is made less than the thickness of the cornea, then the corneal reflex will lie outside the optical section and will be eliminated from the image. For example, if the cornea thickness is 0.5 mm, a practical value for h is 0.35 mm. If a minimum angle θ_n of 6° is assumed, equation 1 requires that the slit image width be 37 μm.

The microscope has been adapted to perform

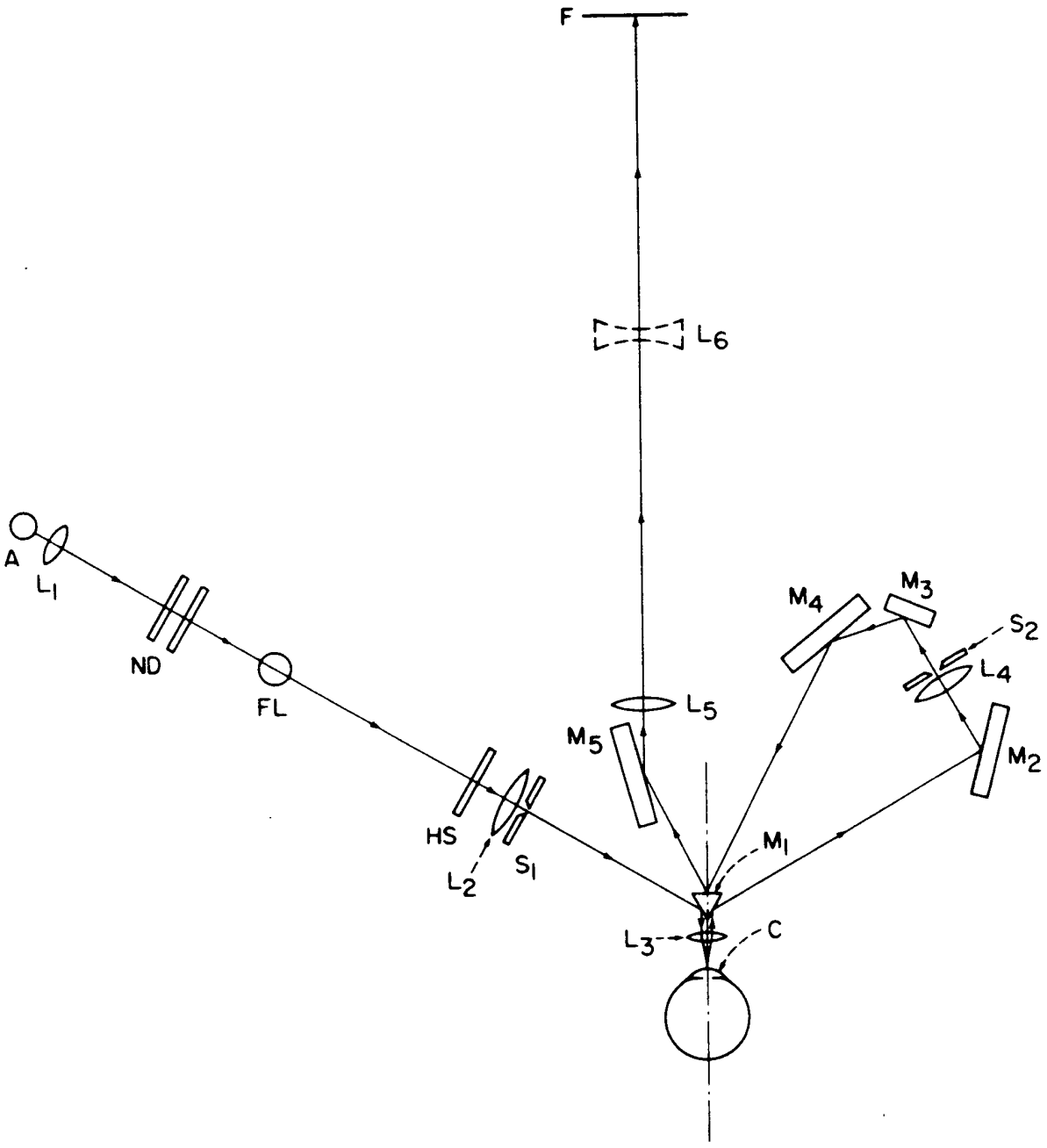


Fig 1. Schematic optical layout of the scanning mirror microscope, clinical model. A: Arc lamp, Xe, 75W; L_1: condenser lens; ND: neutral density filters (removable); FL: flash lamp, Xe, 375 J maximum; HS: heat screen filter; L_2: relay lens; S_1, S_2: slits; M_1: oscillating 3-facet mirror; L_3: objective lens; C: cornea; $M_{2,3,4,5}$: stationary mirrors; L_4: field lens; L_5: image forming lens; L_6: optional negative lens for image magnification; F: film plane.

in either the contact or the non-contact mode. The non-contact objective, a Leitz 25×, NA 0.35, has a 13-mm working distance and is provided with an opaque central stop appropriate for use with human corneas. The contact objective is the Nikon dipping cone objective, 20×, NA 0.33. When the width of slits S_1 and S_2 are set appropriately, the change from contact to non-contact operation can be made merely by changing objectives.

The non-contact mode has the obvious advantages of reducing patient apprehension, avoiding the creation of folds, and eliminating the possibility of a corneal abrasion due to contact. The disadvantage is that patient eye movement can make focusing and photography

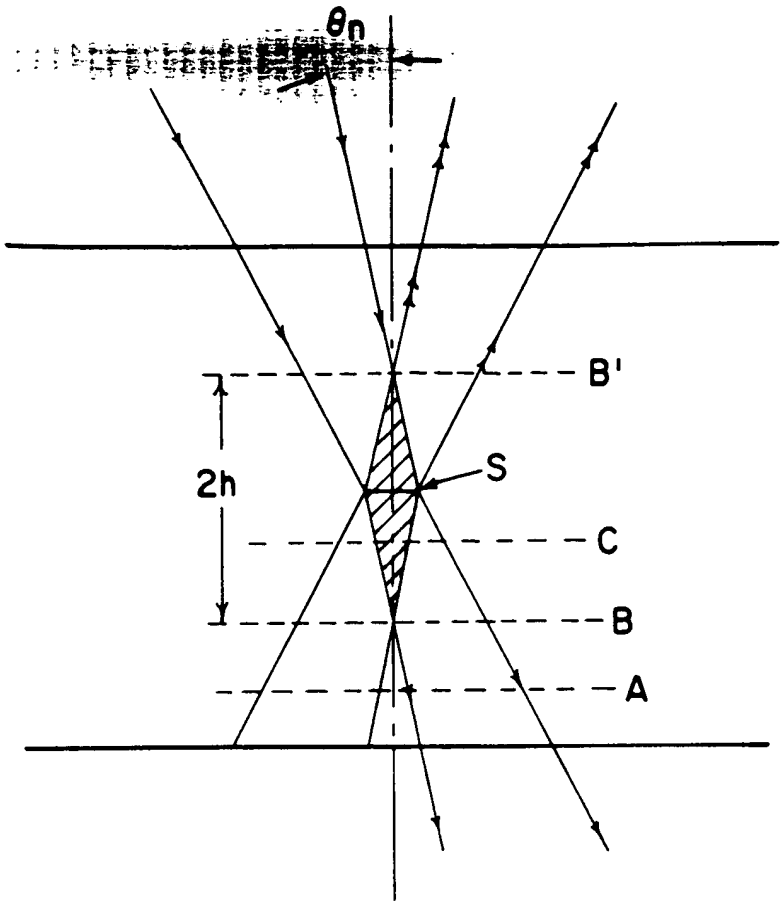


Fig 2. Diagram indicating the formation of the optical section within the specimen. S: image of the illuminated slit S_1; θ_n: minimum angle of the illuminating light rays; A: a plane outside the optical section; B, B': planes between which lies the optical section; 2h: the thickness of the optical section. Reprinted with permission from reference 6.

difficult. The contact mode insures that once the dipping cone is adjusted, the endothelium will stay reasonably in focus even if the eye moves slightly. And the contact both applanates and aligns an area of the cornea normal to the axis of the instrument, thus assuring specular reflection. The disadvantage of the contact mode is primarily the concomitant manipulation of the cornea.

RESEARCH APPLICATIONS

The wide field and optical sectioning capabilities of the microscope suggest a number of research applications.

The ability to return to a landmark on the endothelial monolayer is substantially enhanced by the wide field. Endothelial wound healing can therefore be followed in vivo using time lapse photography. In Fig 5 the recently inflicted wound is seen as an area devoid of endothelial cells. After two hours the rabbit was reanesthetized, placed under the microscope, and the wound area was relocated. Fig 6 illustrates the status after two hours. Small changes are seen near the margins of the wound. After four hours the situation was as seen in Fig 7. And at the end of seven hours, substantial changes were apparent, as shown in Fig 8. This time-lapse technique should provide data complementary to that generated by the techniques employing sacrifice of the animals and histology.

Results of optical sectioning are illustrated in Figs 9, 10, 11, and 12. A 40× NA 0.75 objective was used. Fig 10 was obtained only 25 μm anterior to Fig 9, and yet the endothelial cell pattern is entirely absent from Fig 10. The structures seen are keratocytes, very similar in appearance to those photographed by Maurice[7] and Gallagher and Maurice[8] using a scanning slit microscope. In Fig 11 a different set of keratocytes is seen, 75 μm anterior to those in Fig 10. Finally, in Fig 12, the epithelium is seen, again without scattered light from other levels within the cornea.

CLINICAL APPLICATIONS

As intraocular surgical techniques become more advanced, the maintenance of a viable endothelial monolayer during the postoperative

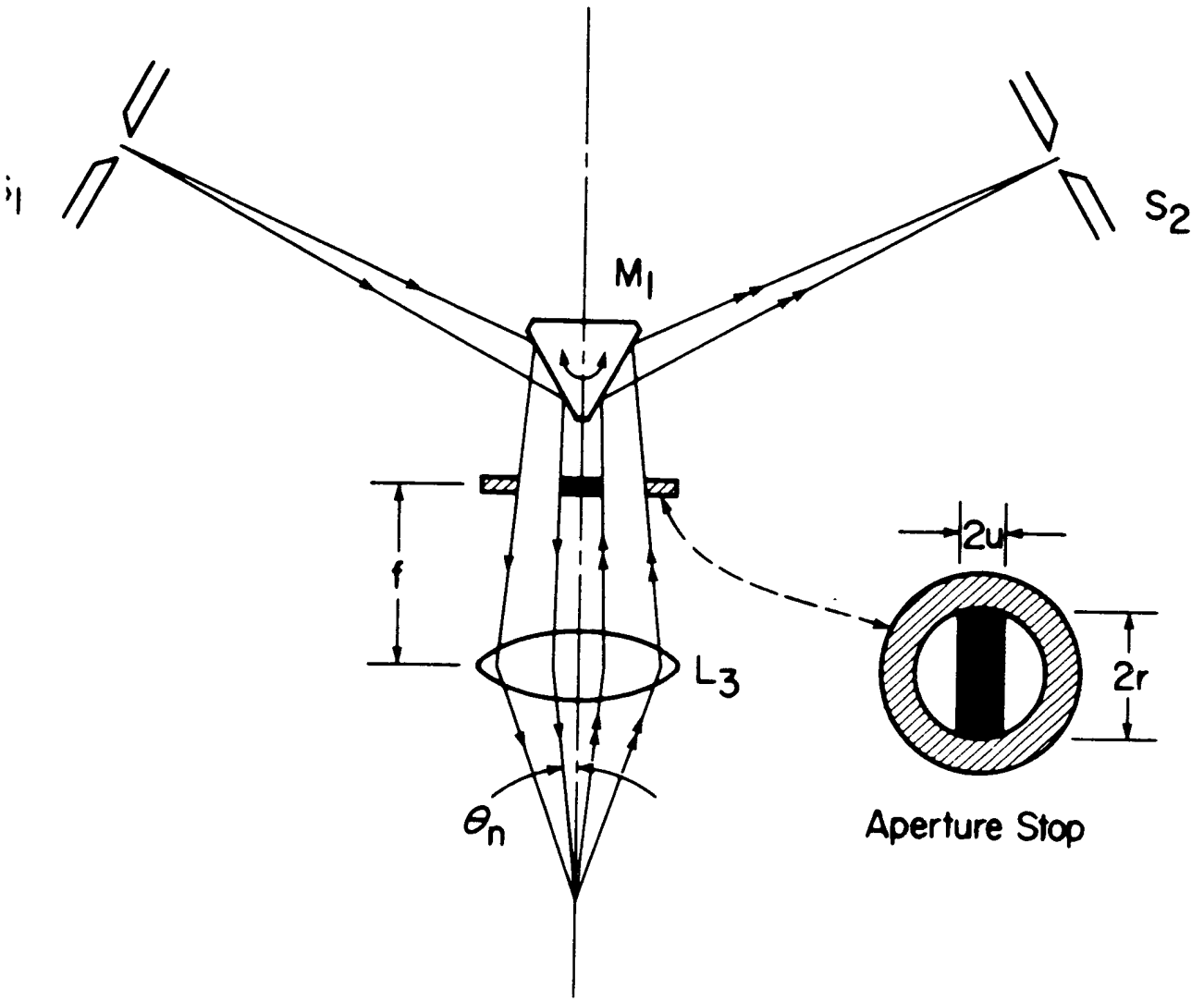


Fig 3. Detailed optical diagram showing the position and configuration of the opaque central strip used to establish the minimum angle θ_n of illumination and imaging rays.

period takes on new importance. Intraocular lens implantation, anterior segment vitrectomy, and glaucoma filtering surgery all carry the risk of significant loss of endothelial cells leading to corneal decompensation. This realization has led to expanded efforts to visualize the endothelium preoperatively to assess its reserve capacity, and then to follow postoperatively for evidence of trauma.

The large field of cells seen with the wide field specular microscope facilitates this assessment by eliminating the sampling characteristics inherent with the small field of existing specular microscopes. Not only does the wide field make cell counting easier and more accurate, but it also allows the viewer the opportunity to appreciate variations in cell size and shape over this greater area.

The wide field has also been used to follow changes in the endothelium over a period of time. Patients with Fuchs' dystrophy are checked periodically for signs of progression of the disease. Endothelial guttata can be more precisely characterized and quantified, which may some day lead to a better understanding of the nature of this dystrophy.

Corneal grafts are also followed to assess their viability. A method for thorough examination of the donor cornea endothelium preoperatively will be described in a later publication. Following the transplant, repeat examinations provide a time lapse history of the normal as well as any abnormal events following transplantation.

An example of normal human endothelium is given in Fig 13. With the large field, cell counting is inherently more accurate than is possible with the conventional small field.

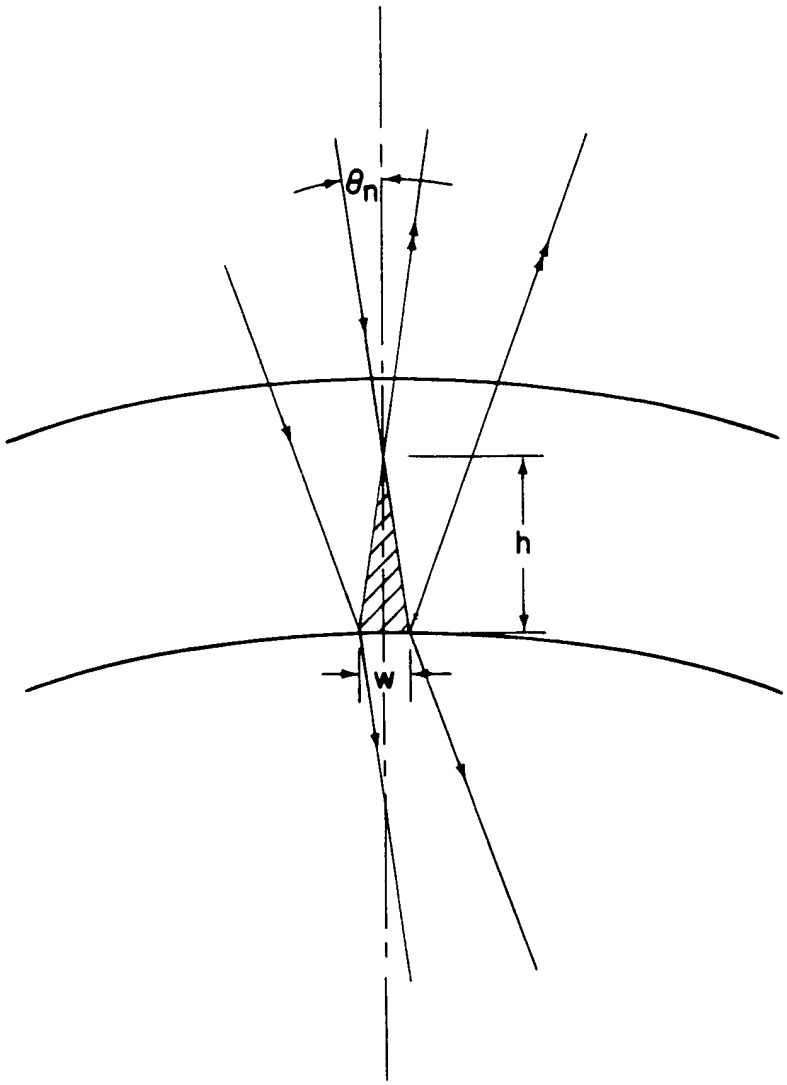


Fig 4. The optical section diagram for non-contact observation of the endothelium. Reprinted with permission from reference 6.

Fig 14 is a non-contact specular micrograph[9] of the endothelium of a four-year postoperative corneal graft. The same cornea, when examined with the conventional dipping cone (contact) objective, exhibited a large number of folds.

DISCUSSION

For a variety of reasons, conventional in vivo endothelial microscopy has been a challenge, considering factors such as corneal haze, variations in corneal thickness, eye movement, and the occasional presence of folds in Descemet's membrane. Wide field microscopy eases these problems, so that the endothelium is more readily focused, examined, and photographed. For example, if stromal haze or folding obscures a small region, the endothelium can still be seen in the remainder of the large field. It is observed that pressure of the dipping cone on the adult cornea increases the number of folds. With the wide field it is possible to monitor the field even in the presence of eye motion, and to reduce the pressure until the optimum fold-free field is obtained.

Two technical considerations limit the size of the image on the film. First, if a 0.9-mm field is to be imaged on the 24-mm wide format of the standard 35-mm film, the magnification is limited to about 26×. Second, the amount of light available at present also limits the magnification to approximately the same value. However, when greater magnification is desired, it can be achieved by inserting a negative lens in the photographic system. The amplitude of the scanning mirror is then reduced to keep the image illuminance at the level needed for film exposure. The advantage gained is that the cell image size is then larger relative to the film grain size, resulting in better resolution of cell detail.

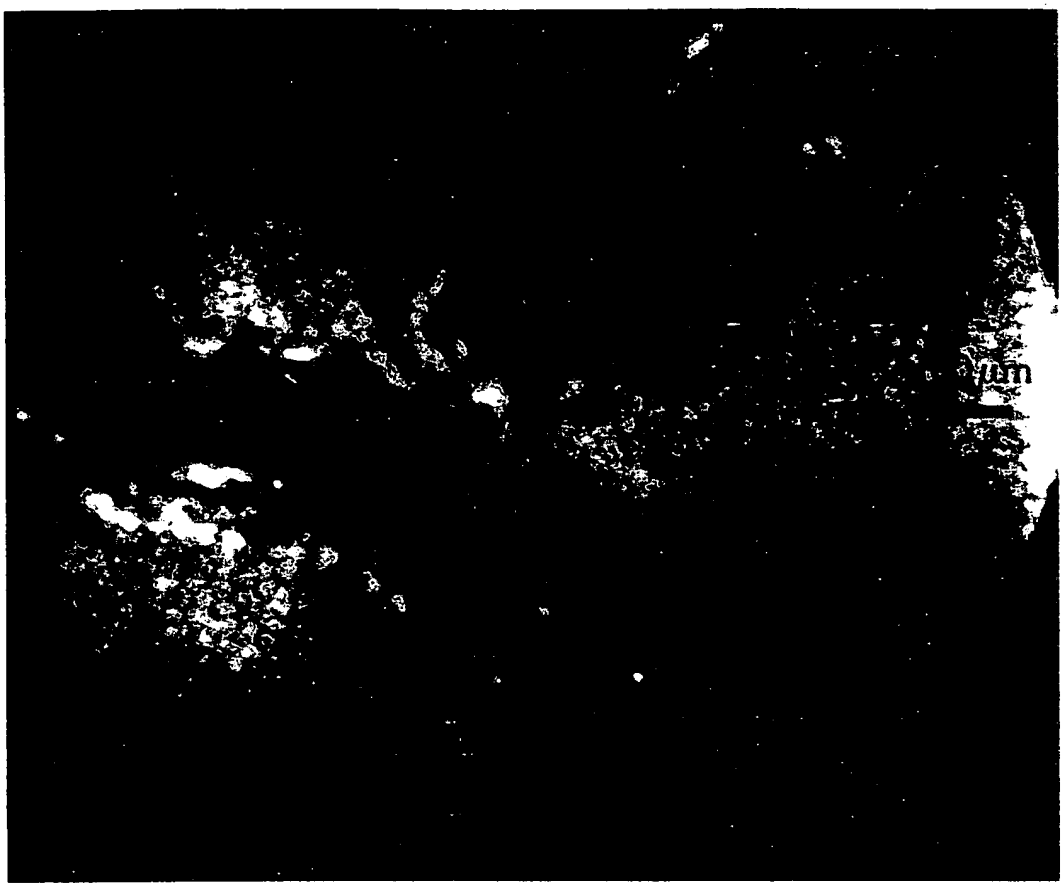


Fig 5. Rabbit cornea immediately after wounding. The implement was a blunt spatula, drawn gently across the endothelial surface.

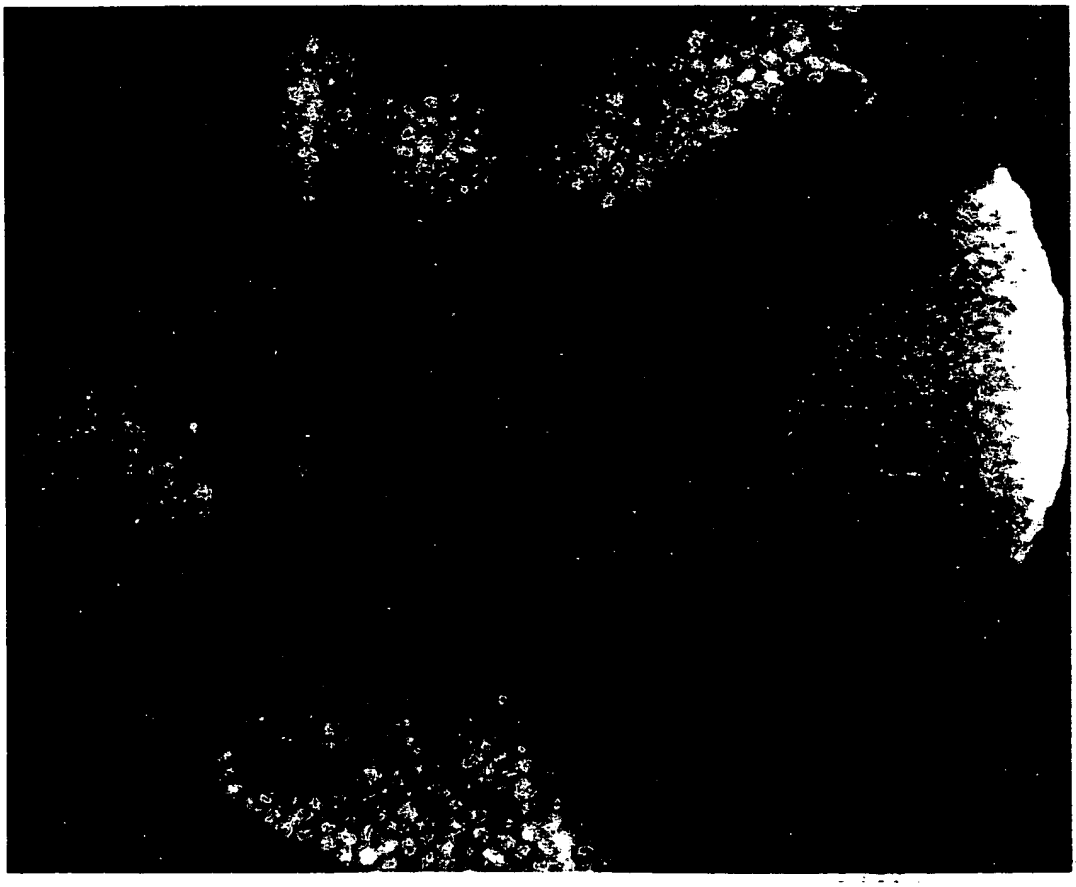


Fig 6. The wounded area two hours later.

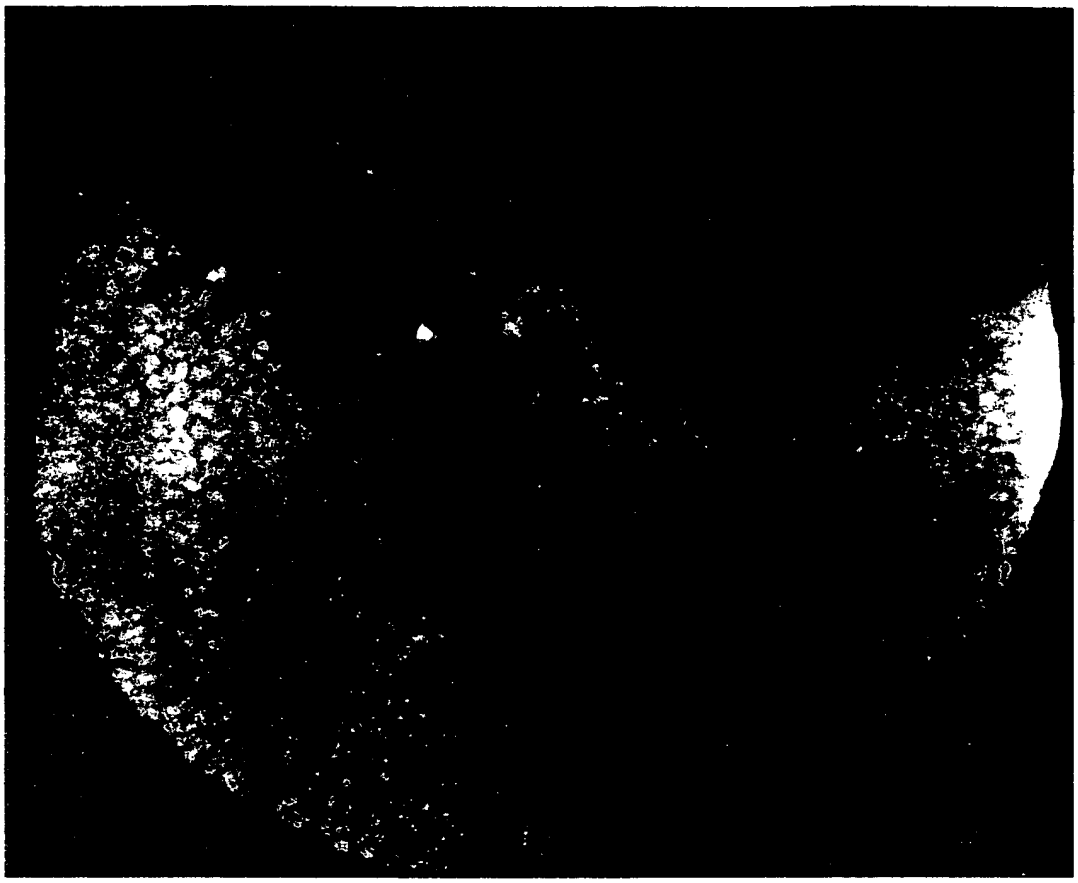


Fig 7. Four hours later than Fig 5.



Fig 8. Seven hours after wounding.

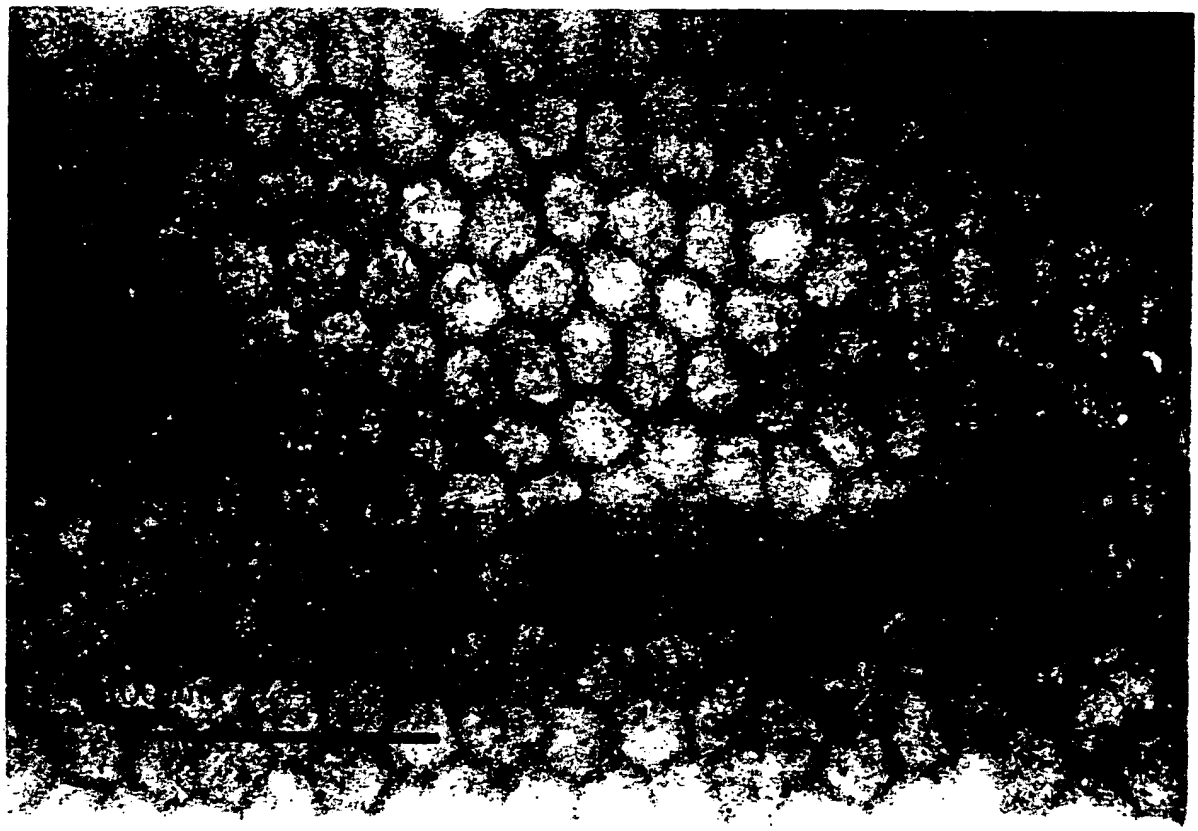


Fig 9. Rabbit endothelium (×40). Reprinted with permission from reference 6.



Fig 10. Microscope focused 25 μm anterior to the field of Fig 9. Reprinted with permission from reference 6.



Fig 11. Microscope focused 100 μm anterior to the field of Fig 9. Reprinted with permission from reference 6.

Laing et al[10] have documented and interpreted a number of abnormalities observed in the endothelium. With the greater uniformity of exposure and contrast obtainable using the scanning mirror microscope, interpretation of certain types of abnormalities should be easier and more accurate, eg, those abnormalities involving variations in brightness within a cell, or from cell to cell across the field.

While only a few applications have been



Fig 12. Epithelial cells 370 μm anterior to Fig 9. Reprinted with permission from reference 6.

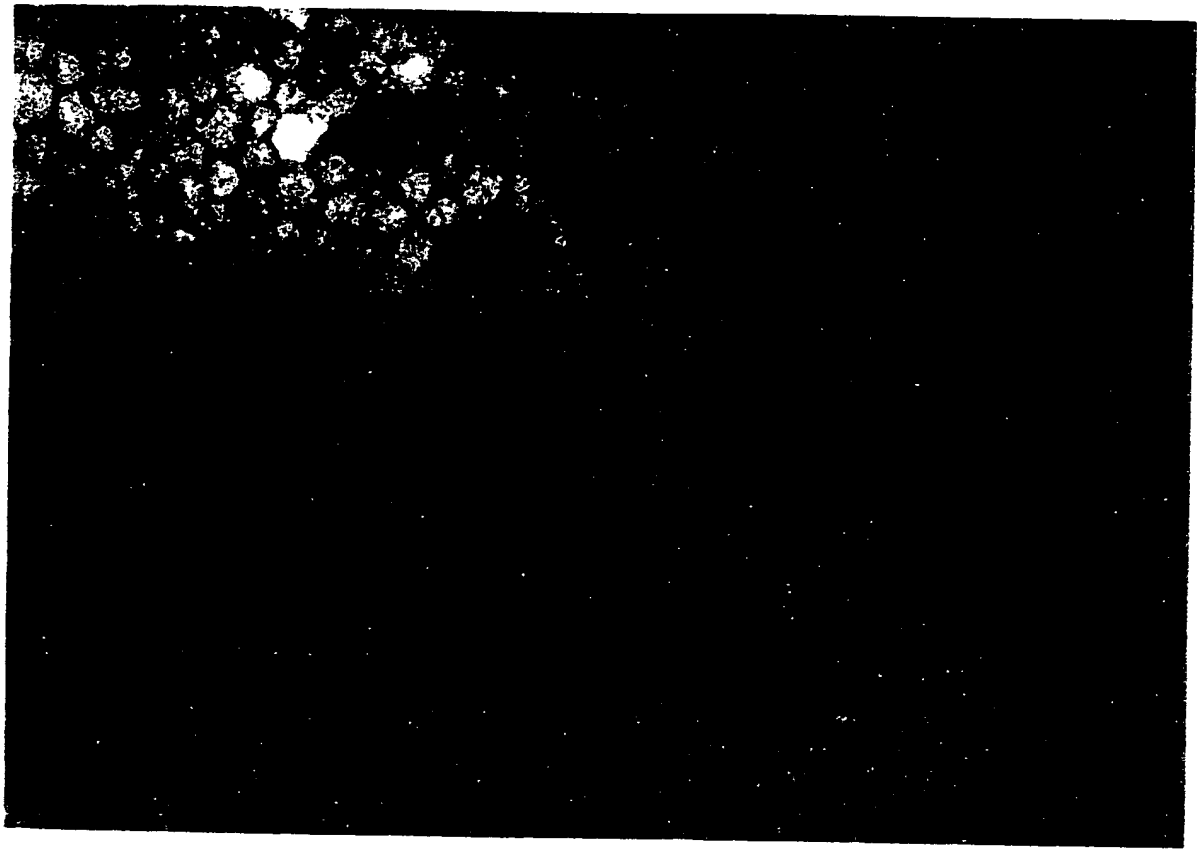


Fig 13. Normal human endothelium.

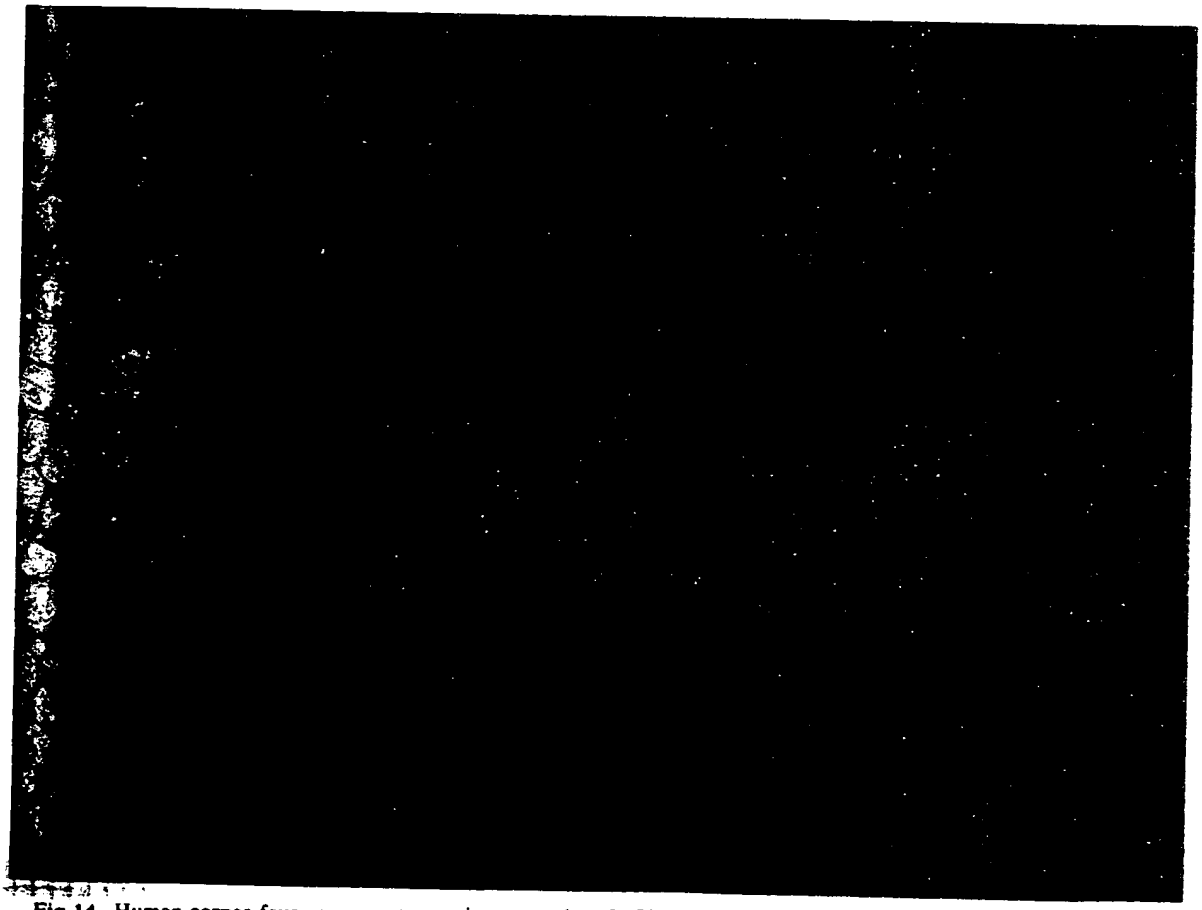


Fig 14. Human cornea four years postoperative corneal graft. Photomicrograph taken with the non-contact objective.

explored to date and are only briefly reported, it is clear that the scanning mirror microscope has certain advantages over the conventional specular microscope.

Qualitative advantages

1. More uniform image brightness and contrast
2. Variation in cell size is readily assessed
3. Endothelial layer topography is more easily evaluated
4. Relocation of a landmark is relatively easy

Quantitative advantages

1. 10 to 25 times larger field
2. More accurate cell counts
3. Fewer photographs are required to obtain a representative sample of endothelial status.

Applications are expected to include: (1) Preoperative and postoperative evaluation of penetrating keratoplasty, intraocular lens implants, cataract surgery, glaucoma surgery, and other surgery in which the endothelium can be affected; (2) Eye bank donor cornea evaluation; and (3) Corneal disease diagnosis and management.

ACKNOWLEDGMENTS

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